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RESEARCH ARTICLE

Disinfection of Gilthead Sea Bream *(Sparus aurata)*, Red Porgy *(Pagrus pagrus)*, and Common Dentex *(Dentex dentex)* Eggs from Sparidae with Different Disinfectants

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Summary

The aim of this study is to determine suitable conditions for egg disinfection of gilthead sea bream *(Sparus aurata)*, red porgy *(Pagrus pagrus)*, and common dentex *(Dentex dentex)* with glutaraldehyde, hydrogen peroxide and iodine. The eggs were disinfected with 200 ppm glutaraldehyde (T= 2, 4, 8, 16 min), 300 ppm hydrogen peroxide and iodine (T= 5, 10, 15, 20 min) at four different contact times. The hatching rate was determined in triplicates for each treatment. Also, bacteria colonies were counted on tryptic soy agar (TSA) and thiosulphate citrate bile salt sucrose agar (TCBS). At the end of the experiment, bacterial load and hatching rate were assessed together. For glutaraldehyde treatment, optimal hatching rates and bacterial colonies were estimated as CT (Disinfectant concentration in $mg.l^{-1}$* exposure time in minute) 800 for sea bream eggs, CT 1600 for red porgy eggs and CT 800-1600 for common dentex eggs. Also, for hydrogen peroxide treatments, they were determined as CT 3000 for sea bream and red porgy and CT 4500 for common dentex. Finally, in iodine treatments, these parameters were measured as CT 4500-6000 for all species' eggs. However, in our study, it is clear that the glutaraldehyde showed the optimum disinfection effect against the microorganisms because the bacteria could be eliminated completely.

Keywords: *Egg disinfection, Sparus aurata, Pagrus pagrus, Dentex dentex*

Sparidae Familyasından Çipura *(Sparus aurata)*, Fangri *(Pagrus pagrus)* ve Sinarit *(Dentex dentex)* Yumurtalarının Farklı Dezenfektan Maddelerle Yüzey Dezenfeksiyonu

Özet

Bu çalışmada çipura *(Sparus aurata)*, fangri *(Pagrus pagrus)* ve sinarit *(Dentex dentex)* yumurtalarının glutaraldehit, iyot ve hidrojen peroksit ile en uygun dezenfeksiyon koşullarının tespit edilmesi hedeflenmiştir. Yumurtalar 200 ppm glutaraldehit ile (2, 4, 8, 16 dak.), 300 ppm hidrojen peroksit ve iyot ile (5, 10, 15, 20 dak.) dört farklı sürelerde dezenfekte edilmiştir. Açılım oranı her uygulamada üçer paralelde tespit edilmiştir. Ayrıca uygulamalar sonrasında TSA (tryptic soy agar) ve TCBS (thiosulphate citrate bile salt sucrose agar) besiyerlerine ekimler yapılarak gelişen bakteri kolonileri sayılmıştır. Denemelerin sonunda bakteri gelişimleri ve açılım oranları birlikte ele alındığında, glutaraldehit ile dezenfeksiyonda çipura yumurtaları için KS (Konsantrasyon*Süre) 800 (200 ppm*4 dak.; 18°C), fangri yumurtaları için KS 1600 (200 ppm*8 dak.; 18°C), sinarit yumurtaları için KS 800-1600 (200 ppm*4-8 dak.; 18°C) değerlerini, hidrojen peroksit uygulamasında çipura ve fangri yumurtaları için KS 3000 (300 ppm*10 dak.; 18°C), sinarit yumurtaları için KS 4500 (300 ppm*15 dak.; 18°C) değerlerini ve iyot ile dezenfeksiyonda ise tüm türlere ait yumurtalarda KS 4500-6000 (300 ppm*15-20 dak.) değerlerini önermekteyiz. Bununla birlikte mikroorganizmaların tam anlamıyla yok edilmesinde en büyük etki glutaraldehit dezenfeksiyonlarında elde edildiğinden, çalışma kapsamında en uygun dezenfektan maddesinin glutaraldehit olduğu açıktır.

Anahtar sözcükler: *Yumurta dezenfeksiyonu, Sparus aurata, Pagrus pagrus, Dentex dentex*

INTRODUCTION

In hatchery conditions, microbial contamination of fish eggs can be problematic, as the egg surface appears to be an attractive substratum for microorganisms [1] and can act to transfer potentially pathogenic bacteria from parent to offspring [2]. Also, egg quality might be relatively increased and contamination of pathogen microorganisms

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be prevented by the disinfection of eggs.

The importance of egg disinfection has gained recognition and researches on the eggs of various species have increased considerably in the recent years [3-5].

On the other hand, it is well known that a higher survival rate and larval quality strongly depend on effective broodstock management and egg quality for both intensively cultured sea bream *(Sparus aurata)* and candidate species such as red porgy *(Pagrus pagrus)* and common dentex *(Dentex dentex)*. Therefore, determination of optimal concentration of disinfectants specifically for each cultured species is very important. So far limited studies have been done on sea bass and sea bream [6,7] but there is no information on other candidate species such as red porgy and common dentex. The main purpose of this study is to determine the optimal exposure time as CT value on the hatchability of sea bream, red porgy and common dentex eggs and the effects of these disinfectants on pathogen microbial activity such as Vibrio spp. and total bacterial loading in tryptic soy agar (TSA) and thiosulphate citrate bile salt sucrose agar (TCBS) in different periods.

MATERIAL and METHODS

Broodstock and Egg Incubation

Gilthead sea bream (12 females 2.6 kg mean weight; 12 males, 1.5 kg mean weight) were selected from wild breeders and stocked in separate 18 m³ tank with a seawater supply of 35 l min^{-1}. Frozen cuttlefish *Sepia officinalis L.,* Leander squilla *Palaemon elegans* L. deepwater rose shrimp *Parapaneaus longirostris* L. and common octopus *Octopus vulgaris* L. were provided daily as the primary food source. The fish were subjected to natural photoperiod of natural rearing seasons. No hormonal treatment was applied to breeders and spawning occurred spontaneously. Broodstock spawn naturally under culture conditions at 16-18°C for sea bream.

Red porgy and common dentex broodstock were caught and acclimated for 2 years in hatchery conditions. 6 females (1.2±0.3 kg mean body weight) and 12 males (0.7±0.2 kg mean weight) as well as 8 females (2.5 kg mean weight) and 8 males (1.4 kg mean weight) respectively were kept indoors in a separate 10 m³ polycarbonate tank under natural photoperiod. Untreated sea water or thermal control was supplied continuously to the tank at the rate of 30 l min^{-1}.

In order to reduce stress and injuries, manipulation and stripping of fish were conducted before and during the spawning season. After the first spawning, fertilized eggs spawned by fish group were collected in egg collectors of each broodstock tank and emptied every 15 min. Viable buoyant eggs were separated from the dead sinking ones. All treatments were done with newly fertilized eggs or at a maximum 2 cells stage. A total of 1 g of eggs was used for each treatment in triplicate.

Disinfection and Microbial Analysis

For disinfection of eggs's surfaces, three different chemicals; glutaraldehyde ($HCO(CH_2)_3CHO$), iodine (Polyvinylpyrrolidone) and hydrogen peroxide (H_2O_2) were used. All erlen meyers and other glass materials used for disinfection were autoclaved (115°C, 45 min, 1.5 atm) either filled with sea water or empty. Firstly, glutaraldehyde (25% v/v, Merck) 200 ppm, H_2O_2 (50% v/v, Merck) and PVP iodine (10% v/v, Argent Chemical Laboratory, USA) 300 ppm were added to erlen mayers (500 ml with steril water) and disinfection solutions were prepared. Only buoyant eggs collected were immersed in these solutions and were agitated gently to ensure that the entire surface was exposed to chemichals. The ratio egg volume/disinfection volume was 1:500. Contact times for glutaraldehyde were 2, 4, 8 and 16 min, for H_2O_2 and iodine were 5, 10, 15 and 20 min (at triplicate). The product CT (concentration * time) was taken into account for comparison of treatments with different combinations of C and T [6].

The bactericidal effect of glutaraldehyde, hydrogen peroxide and iodine was checked on eggs. After treatment, for each time with glutaraldehyde in autoclaved seawater (without treatment for control groups), the eggs were rinsed by three successive baths (1 min each) in sterile seawater and placed in tubes with 10 ml of sterile Letheen solution (Difco TM) that neutralises glutaraldehyde. Hydrogen peroxide and iodine treatment was only washed with sterile sea water for neutralizing and placed in tubes with 10 ml sterile seawater. After neutralizing process, each tube was vigorously shaken for 30 sec, kept without agitation for 15 min, shaken again for 30 sec and kept 1 h without agitation. One ml of solution was transferred into a tube containing 10 ml of sterilized seawater at 17 ppt salinity [6]. Samples for bacterial growth detection were cultured on TSA and TCBS supplemented with 1.5% (w/v) NaCl. Inoculations were performed in triplicate on TSA (total bacteria) and TCBS (Vibrio spp.) agar. Bacterial colonies were counted after an incubation time of 3 days at 20°C as cfu (colony formation unit).

After disinfection, the treated eggs were placed in single incubators (Pyrex beakers) according to treatments, covered with nets with a mesh size of 300 µm. Incubation volume was 1:1000 (1 g egg lt^{-1}). The incubators were placed in a 10 liter volume tank. Water flow rate was

adjusted in a way to change 10% of the total volume of the tanks in an hour and dead eggs were removed immediately. Water temperature was kept at 18±0.2°C for sea bream, red porgy and common dentex. Aeration rate was applied as 40 l min^{-1}. Oxygen saturation, salinity and pH were recorded as 90%, 38‰ and 7.9, respectively. Also, ammonia and nitrite were always P<0.01 mg l^{-1}. Additionally, the disinfectant treatments were measured as CT units.

Statistical Analysis

Results are given as mean±SD. The variance homogeneity of the data was performed using Levene's test. Survival data was compared by Fisher's chi-square test and bacterial monitoring data was compared by one-way ANOVA [7], followed by Kruskall-Wallis multiple range test when significant differences were found at a 0.05 level. All measurements were carried out in triplicate.

RESULTS

Glutaraldehyde Treatment

Gilthead sea bream: Bacterial colonization of TSA plates for sea bream eggs was counted as 102 cfu ml^{-1} at CT 400 value (P<0.05). Also, no bacterial colonization was seen at CT 800, 1600 and 3200 values(P>0.05). However, these values for the control group were found as 1751 cfu ml^{-1} (P<0.05) *(Table 1)*. Although, no bacterial colonization was observed at TCBS plates, it was 2008 cfu ml^{-1} in the control group (P<0.05) *(Table 2)*.

Hatching rate of sea bream eggs were found as 94.67±3.96% at 18°C in CT 400. The best survival rate was determined in CT 800 value as 94.73±3.93%. Also, they were calculated as 83.50±2.43% and 49.33±3.15% at CT 1600 and 3200, respectively. Survival rate of control group was found as 92.13±3.93%. Moreover, CT 400 and 800 values were significantly different from CT 1600 and 3200 values (P<0.05). Besides, there were no significant differences between survival rates of CT 400 and 800 values according to the control group (P>0.05). However, survival rates were significantly different between CT 1600 and 3200 values (P<0.05), no significant differences were found between CT 1600 and control group (P>0.05) *(Fig. 1)*.

Red porgy: Bacterial colonization was found in TSA plates as 417 and 337 cfu ml^{-1} for CT 400 and 800 value for red porgy eggs, respectively, but no colonization was seen at CT 1600 and 3200 values *(Table 1)*. Additionally, 1883 cfu ml^{-1} was counted for control group in TSA plates (P<0.05). Although, bacterial colonization was not counted in TCBS plates among all groups, it was determined to be 783 cfu ml^{-1} in control group (P<0.05) *(Table 2)*.

Survival rate of red porgy eggs was calculated as 91.26±1.28%, 91.32±0.99% and 93.04±3.03% at CT 400, 800 and 1600 values, respectively. The lowest survival rate was determined at CT 3200 value as 50.05±6.3%. Also, the survival rate of the control group was determined as 87.32±1.29% at CT 1600 value. Also, it was found significantly different than the other treatment groups (P<0.05) *(Fig. 1)*.

Common dentex: The most intensive bacterial colonization was observed at CT 400 (140 cfu ml^{-1}) in common dentex eggs and also colonization was observed as 20 cfu ml^{-1} at CT 800. No colonization was observed at CT 1600 and 3200 value, but in control group it was observed as 1570 cfu ml^{-1} (P<0.05) *(Table 1)*. Moreover, no bacterial colonization occurred at TCBS plates, in control group it was 1982 cfu ml^{-1} (P<0.05) *(Table 2)*.

Survival rate of common dentex eggs was determined as 75.86±4.15% at CT 400 value. The highest survival rate was observed at CT 800 (82.24±1.36%) and the lowest survival rate was observed at CT 3200 (46.96±4.90%). Moreover, survival rate of control group was calculated as 59.04±2.48%. Also, CT 400, 800 and 1600 values were significantly different from CT 3200 and control group (P<0.05). Besides, no significant differences were found between CT 3200 and control group *(Fig. 1)*.

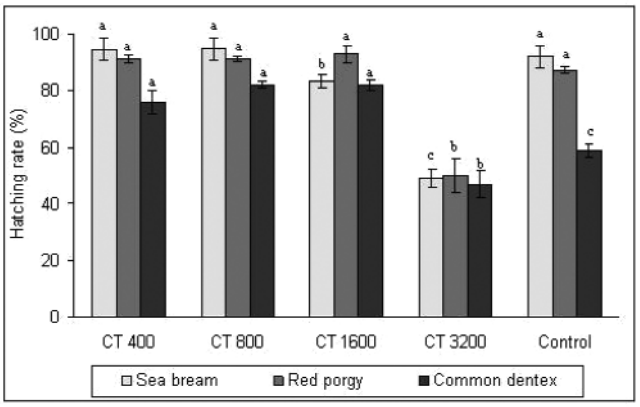

Fig 1. Hatching rates of sea bream, red porgy and common dentex egg with glutaraldehyde treatment

Şekil 1. Çipura, fangri ve sinarit yumurtalarının glutaraldehit uygulamasındaki çatlama oranları

Hydrogen Peroxide Treatment

Gilthead sea bream: After surface disinfection of sea bream eggs with hydrogen peroxide, in TSA plates bacterial colonization was counted as 413, 83, 10 and 7 cfu ml^{-1} at CT 1500, 3000, 4500, and 6000, respectively. Also, 1373 cfu ml^{-1} colony was seen in control group of hydrogen peroxide treatment. The values obtained in CT 1500, 3000, 4500 and 6000 were found significantly different from the control group (P<0.05). Additionally,

colonization was not observed statistically different between CT 3000, 4500 and 6000 values (P>0.05). Also, values between CT 1500 and 3000 and others (CT 4500, 6000 and control group) were found statistically different (P<0.05) *(Table 1).*

In TCBS plates, colonization was determined as 60, 10 and 1170 cfu ml^{-1} for CT 1500, 3000 and control group, respectively. In addition, no bacterial colonization occurred at CT 4500 and 6000 values. Also, CT 1500 value was defined statistically different from other groups (P<0.05). Thus, control group was significantly different from all groups (P<0.05) *(Table 2).*

The highest hatching rate in sea bream eggs disinfected with H_2O_2 was defined as 96.73±0.40% at CT 1500. Survival rates were calculated as 94.73±0.56%, 80.53±0.75%, 55.80±0.80% and 94.60±0.95 % at CT 3000, 4500, 6000, and control group, respectively. Besides, survival rates between control group and CT 1500 and 3000 values presented insignificant similarities (P>0.05). In addition, values of these groups (CT 1500, CT 3000 and control group) were found different from CT 4500 and CT 6000 values (P<0.05). Also, CT 4500 and CT 6000 treated groups were found significantly different than the control group (P<0.05). The effects of H_2O_2 on egg disinfection and survival rates are presented in *Figure 2.*

Red porgy: After surface disinfection of red porgy eggs with hydrogen peroxide, in TSA plates bacterial colonization was counted 1283, 343 and 13 cfu ml^{-1} at CT 1500, 3000 and 4500 respectively. On the other hand, no bacteria colonization was found in CT 6000 value. Also, in control group, 1630 cfu ml^{-1} hydrogen peroxide colony was detected. All experimental groups were found statistically different (P<0.05). It was observed that bacterial colonization in CT 6000 value was prevented (P<0.05) *(Table 1).*

For bacterial colonization in TCBS plates, colonization occurred as 130, 10 and 7 cfu ml^{-1} for CT 1500, 3000 and 4500 values, respectively. In addition, no bacterial colonization was observed at CT 6000 value. Also, in control group, 993 cfu ml^{-1} colonies were counted. CT 1500 value was defined statistically different from CT 3000-4500 and control group (P<0.05). Although, there were no significant differences at CT 1500, 3000 values (P>0.05), control group was determined statistically different from all groups (P<0.05) *(Table 2).*

The highest survival rate of red porgy eggs were at CT 1500 value (97.18±0.34%), but it was not found statistically different compared with control group (95.34±0.82%) and CT 3000 (95.46±0.49%) (P>0.05). At the same time, hatching rate at CT 4500 value decreased (83.22±1.67 %) and the lowest value (49.71±3.11%) was calculated for CT 6000 (P<0.05) *(Fig. 2).*

Common dentex: At the end of the experiment, in TSA plates bacterial colonization was determined as 850, 973, 410, and 340 cfu ml^{-1} colonies for CT 1500, 3000, 4500 and 6000 for common dentex eggs, respectively. In control group, 1930 cfu ml^{-1} colony was defined (P<0.05) *(Table 1).* Control group was found significantly different from all groups (P<0.05). On the other hand, colonization at CT 1500 and 3000 values was counted as 707 and 17 cfu ml^{-1}, respectively and no bacteria colonization was found in other groups in TCBS plates. It was observed that 1553 colony occurred in control group (P<0.05). CT 1500 was defined significantly different from all groups (P<0.05) *(Table 2).*

Survival rates of common dentex eggs were determined as 65.38±10.25, 66.77±6.44 and 69.75±5.77% at CT 1500, 3000 and 4500 respectively. The lowest survival rate was found at CT 6000 value (39.54±7.70%). Hatching rate of control group was defined as 62.85±0.95%. The relation between CT 1500, 3000 and 4500 values and control group was not found statistically important (P>0.05). CT 6000 value was determined to be different from other values (P<0.05) *(Fig. 2).*

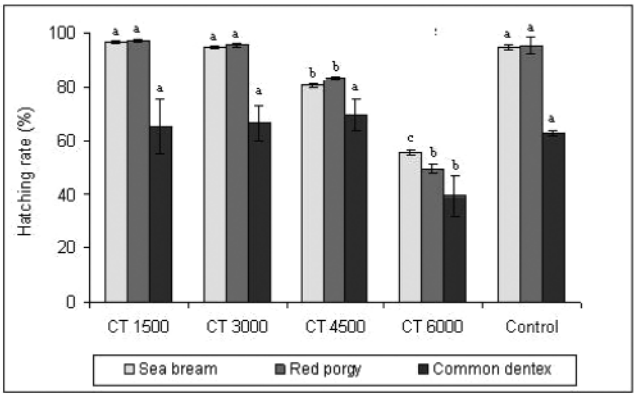

Fig 2. Hatching rates of sea bream, red porgy and common dentex egg with hydrogen peroxide treatment

Şekil 2. Çipura, fangri ve sinarit yumurtalarının hidrojen peroksit uygulamasındaki çatlama oranları

Iodine Treatment

Gilthead sea bream: After surface disinfection of sea bream eggs with iodine, in TSA plates bacterial colonization was defined as 1023, 1207, 807 and 699 cfu ml^{-1} colony at CT 1500, 3000, 4500 and 6000, respectively. Also, 1380 cfu ml^{-1} colony was determined in control group. At the end, there were no significant differences among groups (P>0.05) *(Table 1).* For incubation experiments in TCBS plates, no colonization was counted in all experimental groups; however colonization occurred as 1107 cfu ml^{-1} in control group (P<0.05). Control group was found significantly different from all groups (P<0.05) *(Table 2).*

After incubation, hatching rates of gilthead sea bream eggs were determined as 87.40±1.57, 88.77±2.11, 88.83±1.20, 90.57±3.46 and 89.97±2.12% in CT 1500, 3000, 4500, 6000 and control group, respectively (P<0.05) *(Fig. 3)*.

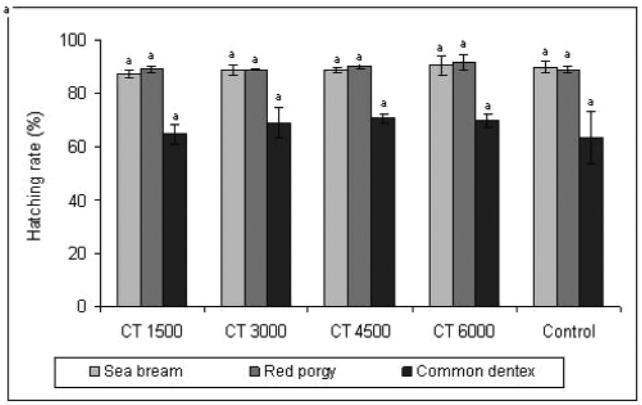

Fig 3. Hatching rates of sea bream, red porgy and common dentex egg with iodine treatment

Şekil 3. Çipura, fangri ve sinarit yumurtalarının iyodin uygulamasındaki çatlama oranları

Red porgy: In surface disinfection of red porgy eggs with iodine, bacteria colonization were determined in TSA plates as 1027, 1037, 433, 330 and 1443 cfu ml^{-1} at CT 1500, 3000, 4500, 6000 (P>0.05) and control group (P<0.05) values, respectively *(Table 1)*. CT 1500 and CT 300 were different from CT 4500, CT 6000 and control group. In TCBS plates, 480 and 447 cfu ml^{-1} colonies were counted at CT 1500 and 3000, respectively (P>0.05). No bacterial colonies occurred at CT 4500 and 6000 values. Also, in control group, 853 cfu ml^{-1} colonies were observed (P<0.05). CT 1500 and 3000 values were found significantly different from other groups (P<0.05). Additionally, similar results were estimated in 10 and 20 min disinfection treatments (P>0.05) *(Table 2)*.

Hatching rates of red porgy eggs were presented close similarity and calculated as 89.13±1.35, 89.05±0.40, 90.37±1.04, 91.87±2.98 and 89.02±1.23% at CT 1500, 3000, 4500, 6000 and control group (P>0.05) *(Fig. 3)*.

Common dentex: Colony numbers in TSA plates were observed as 1180, 1020, 807 and 790 cfu ml^{-1} for each CT values, respectively. Also, 1550 cfu ml^{-1} colonies were counted in control group (P>0.05) *(Table 1)*. In TCBS plates, 993, 480 and 1717 cfu ml^{-1} colonies occurred at CT 1500, 3000 and control group, respectively. No bacterial colonization was determined at CT 4500 and 6000 values. Moreover, CT 1500 and 3000 values were found significantly different from control and other experimental groups (P<0.05). Also, there were significant differences between CT 1500, 3000 and control group (P<0.05). In addition, no significant differences were found between CT 4500 and 6000 values (P>0.05) *(Table 2)*.

The highest survival rate of common dentex eggs was found as 70.65±3.43% at CT 4500 value. Additionally, survival rates at CT 1500, 3000 and 6000 were determined

Table 1. *Bacterial colonization in sea bream, red porgy, and common dentex eggs in glutaraldehyde, hydrogen peroxide and iodine treatment under different CT value (Disinfectant concentration in mg l^{-1}* exposure time in minute) in TSA (cfu ml^{-1}) plate. Means with the same superscript are not significantly different (P>0.05) (C: Control group)*

Tablo 1. *Çipura, fangri ve sinarit yumurtalarının dezenfeksiyonunda kullanılan glutaraldehit, hidrojen peroksit ve iyodin maddelerinin farklı KS değerlerinin (Konsantrasyon, mg l^{-1} * Süre, dakika) TSA besiyerindeki bakteri gelişimine (koloni adedi ml^{-1}) etkileri (K: Kontrol grubu)*

Species	Glutaraldehyde					Hydrogen peroxide					Iodine				
	400	800	1600	3200	C	1500	3000	4500	6000	C	1500	3000	4500	6000	C
Sea bream	102 ^{a}	-	-	-	1751 ^{b}	413 ^{a}	83 ^{b}	10 ^{b}	7 ^{b}	1373 ^{c}	1023 ^{a}	1207 ^{a}	807 ^{a}	699 ^{a}	1380 ^{a}
Red porgy	417 ^{a}	337 ^{a}	-	-	1883 ^{b}	1283 ^{a}	343 ^{b}	13 ^{c}	-	1630 ^{d}	1027 ^{a}	1037 ^{a}	433 ^{b}	330 ^{b}	1443 ^{c}
Common dentex	140 ^{a}	20 ^{b}	-	-	1570 ^{c}	850 ^{a}	973 ^{a}	410 ^{a}	340 ^{a}	1930 ^{b}	1180 ^{a}	1020 ^{a}	807 ^{b}	790 ^{b}	1550 ^{c}

Table 2. *Bacterial colonization in sea bream, red porgy and common dentex eggs in glutaraldehyde, hydrogen peroxide and iodine treatment under different CT value (Disinfectant concentration in mg l^{-1}* exposure time in minute) in TCBS (cfu ml^{-1}) plate. Means with the same superscript are not significantly different (P>0.05) (C: Control group)*

Tablo 2. *Çipura, fangri ve sinarit yumurtalarının dezenfeksiyonunda kullanılan glutaraldehit, hidrojen peroksit ve iyodin maddelerinin farklı KS değerlerinin (Konsantrasyon, mg l^{-1} * Süre, dakika) TCBS besiyerindeki bakteri gelişimine (koloni adedi ml^{-1}) etkileri (K: Kontrol grubu)*

Species	Glutaraldehyde					Hydrogen peroxide					Iodine				
	400	800	1600	3200	C	1500	3000	4500	6000	C	1500	3000	4500	6000	C
Sea bream	-	-	-	-	2008 ^{a}	60 ^{a}	10 ^{b}	-	-	1170 ^{c}	-	-	-	-	1107 ^{a}
Red porgy	-	-	-	-	783 ^{a}	130 ^{a}	10 ^{b}	7 ^{b}	-	993 ^{c}	480 ^{a}	447 ^{a}	-	-	853 ^{b}
Common dentex	-	-	-	-	1982 ^{a}	707 ^{a}	17 ^{b}	7 ^{b}	-	1553 ^{c}	993 ^{a}	480 ^{b}	-	-	1717 ^{c}

as 64.82±5.41, 69.13±1.52 and 69.89±2.32%, respectively. Also, in control group, survival rate was estimated as 63.75±9.67% (P>0.05) *(Fig. 3).*

DISCUSSION

During the last decade, more studies have focused on egg surface disinfection in marine fish such as sea bream [6,8], turbot *(Scopthalmus maximus)* and striped trumpeter *(Latris lineate)* [9], red porgy and white sea bream *(Diplodus sargus)* 11 eggs. In respect to glutaraldehyde treatments, it has been reported that bacterial colonization was prevented with CT 4000 (400 *ppm*10* min) and higher concentrations than this value in striped trumpeter and turbot eggs [5,6]. Nevertheless, it is estimated that bacterial colonization was stopped at CT 800 (200 *ppm*4* min) value in surface disinfections of sea bream eggs [6]. However, it is reported that glutaraldehyde treatment 200-400 mg l^{-1} for 5 min for red porgy and 400 mg l^{-1} for 5 min for white sea bream inhibited bacterial development while decreasing the hatchability of larvae [11]. In this study, CT 1600-upper and CT 800-upper values prevented bacterial colonization in TSA and TCBS plates, respectively. As described by Roy et al.[12], these differences were derived from temperature, pH and water quality and/or bacteria species on the egg.

Similarly, glutaraldehyde treatment increased larval survival rates of halibut [13]. With respect to chloramines T, iodine and sodium hypochlorite disinfection, the glutaraldehyde presented best results in European plaice *(Pleuronectes platessa),* cod *(Gadus morhua)* and sole *(Solea solea)* eggs [14]. In this study, hatching rates increased in disinfection groups compared to control group in sea bream and red porgy eggs below the lethal dose (CT 3200) for these species.

In addition, egg disinfection could be relatively decreased to microbial activity and also similar findings were reported in some studies [6,10,15]. With respect to common dentex, hatching rates of control group were relatively lower than other species' control. It was considered surprising that these low hatching rates could be caused by microorganisms. Additionally, survival rates of common dentex eggs did not achieve high rates even under optimal disinfection conditions. Especially, the lower hatching rates encountered in candidate species were strongly related to and change with egg quality (genetic characteristics from breeders, feeding factors, abiotical factors, etc.), regional conditions, and microbial activity in culture conditions [9,16].

Besides, negative effects of glutaraldehyde were observed after 8 min (CT 1600) for sea bream, but this value was measured for common dentex and red porgy eggs as 16 min (CT 3200). After 16 min (CT 3200) treatment, embryonic development of sea bream, and red porgy eggs were retarded, and hatching time was elongated compared to control group. In 4 and 8 blastomere stages, blastomere symmetry in both species showed shape deformities and resulted in mortality in morula stage. Also, abnormal blastomere formations (asymmetrical cleavage) are the major factors for egg development [17].

At the end of the experiments, it was observed that some larvae did not completely hatch out and stayed in their chorion at CT 3200 value. As described by Escaffre et al.[6], eventual penetration of glutaraldehyde inside the eggs and direct toxic action on the embryos, one reason explaining glutaraldehyde toxicity for fish egg, have an effect on the egg chorion. Also, it is reported that relatively higher concentrations of glutaraldehyde (CT 16000) caused chorion to harden, elongated the hatching time, and decreased the survival rates in European plaice *(Pleuronectes platessa)* eggs Salvesen [14]. Nevertheless, morphological shape and synchronized larval behavior were not observed between newly hatched larvae of sea bream and red porgy and also hatching took approximate durations in all groups. In this study, mortality of common dentex eggs continued in different embryonic stages (blastomere, morula and gastrulation) at CT 3200 value. Some studies recorded that negative effects are related with the changing of influence rate of glutharaldehyde on tissue type and water temperature [18].

It is well known that water temperature is effective on the duration of CT value. In this study, 200 ppm glutaraldehyde disinfections were applied to 3 different species, and finally CT values were determined as 800 (200 ppm*4 min), 1600 (200 ppm*8 min), 800-1600 (200 ppm*4-8 min) at 18°C for sea bream, red porgy and common dentex eggs. These obtained values were relatively lower than those of cold water species in spite of differences in experimental design.

When it comes to H_2O_2 disinfections of sea bream and common dentex eggs, the obtained results were not in parallel with those of red drum at 300 ppm concentration and 5 min application time (CT 1500) [19]. Similarly, in common dentex eggs, the best hatching rates were obtained at 15 min treatment time and the findings did not present significant differences statistically in TSA plates (P>0.05), although TCBS results were significant (P<0.05). However, bacterial colonization was not restrained in TSA plates even at 300 ppm concentration and 20 min application time. But, in TCBS plates, this development was inhibited only at this treatment dose.

In egg disinfection with H_2O_2, it was observed that there were no individual differences in developmental stages of eggs and newly hatched larvae of sea bream and red porgy. Also, there were no abnormalities in terms of both behavioral and morphological characteristics of newly hatched larvae. In disinfection of red porgy eggs, it was determined that this species presented the worst results in terms of durability and the survival rate decreased by approximately 50% in spite of using a standard dose of disinfectant. Like in glutaraldehyde treatment, abiotical factors and egg characteristics showed descriptive differences in H_2O_2 egg disinfections as described by Douillet and Holt [18]. As a result, it could be suggested that suitable CT values with H_2O_2 disinfection were obtained as 3000 (300 ppm*10 min) for sea bream and red porgy, 4500 (300 ppm*15 min) for common dentex eggs at 18°C, But, bacterial colony development was not restrained completely.

Positive results of egg disinfections with iodophore were reported in red porgy and white sea bream *(Diplodus sargus sargus)* [11], Atlantic halibut *(Hippoglossus hippoglossus)* [13], grouper *(Epinephelus coioides)* [20], sturgeon *(Acipencer stellatus)* [21]. In this study, obtained results from 300 pmm treatment in experimental fishes, Vibrio species were restrained especially in CT 1500-3000 values, and this result was in parallel with other studies which eliminated different pathogen species [20,22]. However, total bacteria loading did not decrease significantly in the current experiment (P>0.05). Generally, iodine had different effects on organisms depending on water quality (pH, organic materials etc.). Toxic effect of iodine increased between 6 and 8 pH values. In this study, fatal effect of iodine was lower in experimental fish than freshwater species, because pH was measured to be over 8 due to usage of sea water. Additionally, fatal effect of iodine commonly occurred at optimum level between 7-7.5 pH values, and it may be correlated with oxidation and halogenization of microorganisms. In several studies in which distilled water was used, iodine restrained various pathogenic organisms even at low concentrations [23]. Also, the effect of disinfectant strongly changed depending on bacterial resistance in environment [23,24]. It is clear that relatively higher concentrations of iodine treatment had a significant effect on the hatchability of the red porgy and white sea bream eggs [7]. In the present study, it was found out that survival rates did not decrease at CT 6000 value in iodine disinfections of sea bream eggs, although the treated dose was relatively higher than it was in other studies. It is commonly known that toxic effect of iodine on organisms depends on the acidity and alkalinity of water. As described for sea bream and common dentex eggs, it was determined that bacteria colonization did not decrease at 15-20 min iodine disinfections but not inhibited total bacteria loading in red porgy. Also, it was defined that disinfection treatment for 5 to 20 min at 300 ppm did not present toxic effects on sea bream, common dentex and red porgy eggs. Additionally, there is no record about the negative effects of iodine on both embryonic development and newly hatched larvae as found in the current experiments. In this study, it is suggested that the best CT values in 300 ppm iodine disinfection were defined as 4500-6000 (15-20 min) for sea bream, common dentex and red porgy at 18°C but it was not found statistically different among groups (P>0.05). It is thought that, relatively higher survival rates could be obtained from relatively higher dosage treatments (even existing microorganisms) in culture conditions as reported by Brown et al.[25].

As a result of these findings, when bacterial load is compared with hatching rate, it is clearly suggested that for glutaraldehyde treatment, hatching rates and bacterial colony were determined as CT 800 (200 ppm*4 min; 18°C) for sea bream eggs, CT 1600 (200 ppm*8 min; 18°C) for red porgy eggs and CT 800-1600 (200 ppm*4-8 min; 18°C) for common dentex eggs, respectively. Also, for hydrogen peroxide treatments, these parameters were determined as CT 3000 (300 ppm*10 min; 18°C) for sea bream and red porgy and CT 4500 (300 ppm*15 min; 18°C) for common dentex. Finally, in iodine treatments, these parameters were measured as CT 4500-6000 (300 ppm*15-20 min) for all species (water temperature was 18°C during egg incubations). However, in our study, it is clear that the glutaraldehyde showed the optimum disinfection effect against micro-organisms because the bacteria could be eliminated completely.

Due to the impossibility of egg preservation alive in present, it is very important to evaluate these eggs in hatcheries by minimal mortality. Also, contamination of pathogen microorganisms could be easily prevented by using disinfection procedures in hatcheries especially during egg transfer from farms to farms. Additionally, optimization and descriptive characteristics of disinfection procedures are also important for international aquaculture in order to prevent these contaminations. Further studies should be focused on the efficacy of using organic materials in safe concentrations and biotechnological approaches to disinfections of fish eggs.

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